OBSTETRICS

Alterations to the maternal circulating proteome after preeclampsia

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OBJECTIVE: The long-term maternal cardiovascular and metabolic implications associated with preeclampsia (PE) include risk of hypertension, heart disease, and metabolic syndrome. The objective of this study was to investigate if a recent history of PE and/or lifetime risk of cardiovascular disease (CVD) was associated with detectable alterations in the circulating maternal proteome.

STUDY DESIGN: Six-month postpartum plasma from PE women (n = 12) and women with uncomplicated obstetrical history (n = 12) were used for analysis. Depleted maternal plasma was analyzed by label-free liquid chromatography-mass spectrometry assay. Identified peptides were searched against the International Protein Index human database version 3.87. Exponentially modified protein abundance indices were used for comparison. Results were analyzed using pathway analysis.

RESULTS: A total of 126 eligible peptides were identified for analysis; 3 peptides were differentially expressed in the PE proteome, and an additional 5 peptides were unique to control subjects and 7 to PE subjects. PE peptide profiles were more strongly associated with markers of coagulation and complement activation compared to controls and mapped more significantly to CVD functions. Stratification of subjects by low (<39%) and high (≥39%) lifetime risk of CVD rather than by diagnosis produced similar findings. Comparison of low-risk controls (n = 6) to low-risk PE subjects (n = 6) found that while similar for body mass indices, blood pressure, and fasting lipid profiles at 6 months postpartum, PE peptide profiles continued to display stronger associations for coagulation and CVD functions. Global network analysis found that unique peptides to low-risk PE subjects were associated with cardiac infarction, CVD, and organismal injury and abnormalities.

CONCLUSION: Markers of CVD and cardiovascular dysfunction are evident in the maternal circulating proteome at 6 months postpartum after PE. Augmentations in circulating peptide profiles occur in patients with previous PE who otherwise do not have clinically measurable cardiovascular risk factors. Our data highlight the need for the implementation of postpartum prevention programs in the PE population and identifies molecules that may be targeted for screening or therapeutic benefit.

Key words: cardiovascular risk, maternal health, preeclampsia, proteome

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Introduction

Preeclampsia (PE) is a hypertensive disorder of pregnancy that poses significant risk of adverse maternal and fetal outcomes. The pathogenesis underlying PE remains poorly understood, although manifestations of impaired endothelial function, oxidative stress, and hypercoagulability bear striking similarity to those observed in states of cardiovascular risk and cardiovascular disease (CVD). Indeed, the long-term maternal cardiovascular and metabolic implications associated with PE are well established and include risk of heart disease, stroke, and the metabolic syndrome.[1,2]

We postulate that the early postpartum period offers a unique window

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of opportunity for the identification and education of high-risk women. A lack of programs providing cardiovascular risk screening based on obstetrical history poses a significant hurdle to risk reduction in this population, however.[3] Development of risk factors for CVD soon after PE, including persistent hypertension, dyslipidemia, insulin resistance, as well as propensity for weight retention, allude to early changes in the biophysical profiles of these women that could be targeted before the development of CVD itself.[4,5] In addition to traditional risk factors, evidence exists for impaired flow-mediated dilation, increased artery intima media thickness, and cardiac remodeling after pregnancies complicated by PE.[6-9]

The use of nontraditional markers of cardiovascular risk to assess states of cardiovascular health in postpartum women remains important in establishing the short- and long-term implications of PE and other hypertensive disorders of pregnancy. Recently, induction of PE-like symptoms via overexpression of soluble fms-like tyrosine kinase (sFlt)-1 in CD1 mice was shown to induce long-term alterations in maternal peptide profiles after pregnancy.[10] Translation of knowledge gained from animal models of PE to human study is critical for the development of preventative strategies aimed at minimizing the burden of PE and CVD on health care systems.

Only a handful of clinical studies have been undertaken in the past 10 years to examine the proteome associated with PE, and the majority have focused on the examination of placental tissue.[11-17] Of those studies examining maternal biofluids, none have attempted analysis of the postpartum circulating proteome in women with a history of PE.[18-23] Given the evidence for a predisposition to CVD after PE, we sought to determine if women who had developed PE during the index pregnancy had detectable alterations in the circulating maternal proteome 6 months postpartum. Furthermore, data on cardiovascular risk profiles were used to examine whether the presence or absence of classic cardiovascular risk factors impacted differences between control and PE peptide profiles.

Materials and Methods

Sample collection

This study was approved by the Queen's University Research Ethics Board. Written informed consent was obtained from all subjects. Plasma samples were collected from women attending the maternal health clinic at Kingston General Hospital at 6 months postpartum. The maternal health clinic is designed to provide postpartum cardiovascular risk screening and counseling to all mothers delivering at Kingston General Hospital with select obstetrical complications, including PE.[24] Biophysical measurements and fasting blood work collected by the clinic are used to generate lifetime cardiovascular risk scores for each patient to help guide maternal cardiovascular risk counseling. Plasma samples included for analysis were from women who either had experienced pregnancies complicated by PE ($n = 12$) or normotensive uncomplicated pregnancies ($n = 12$). PE was defined as the development of de novo hypertension ($\geq$140/90 mm Hg) and proteinuria (>300 mg/24 h or +1 on repeat dipstick). A clinical diagnosis was confirmed by chart review. Individuals with a self-reported history of hypertension, diabetes (including the development of gestational diabetes), kidney disease, CVD, or current smoking were excluded following confirmation by chart review.

Lifetime risk for CVD was calculated for each subject at the maternal health clinic. Calculations for lifetime cardiovascular risk are based on biophysical factors: sex, smoking, total cholesterol fasting glucose, systolic blood pressure, diastolic blood pressure, and antihypertensive usage.[25] Risk estimates are categorical: low risk (<39%) and high risk ($\geq$39%).

Sample preparation for mass spectrometry

Plasma was analyzed for each subject individually. Whole blood samples were collected into EDTA and centrifuged at 1000*g* for 10 minutes within 2 hours of collection. Plasma was isolated and stored in aliquots at −80°C. Samples used for analysis were stored for a maximum of 3 years prior to analysis.

A total of 20 μL of each plasma sample was diluted with 0.7 μL (×10 dilution) of protease inhibitor (Sigma-Genosys, Spring, TX). Whole plasma was depleted Q6 of 14 highly abundant proteins using Agilent Human 14 Multiple Affinity Removal Columns (Agilent Technologies, Santa Clara, CA) according to manufacturer's instructions. Flow-through fractions were concentrated and buffer exchanged to 100 mmol/L ammonium bicarbonate by centrifugal filtration through a 5-kDa MWCO Agilent spin concentrator (Agilent Technologies). High molecular−weight fractions for each sample were collected and a small aliquot used to perform a Bradford total protein assay (Bio-Rad, Hercules, CA).

Depleted plasma samples were denatured with 6 mol/L urea in 150 mmol/L Tris HCl, pH 8.0, and reduced with 20 mmol/L DTT at 37°C for 40 minutes. Samples were then alkylated with 40 mmol/L iodacetamide in the dark for 30 Q7 minutes and diluted 10-fold with 50 mmol/L Tris-HCl pH 8.0 prior to overnight digestion at 37°C with trypsin (Promega, Madison, WI). Digestion was terminated with equal volume 1% formic acid. Samples were desalted with Waters Oasis C18 cartridges (Waters, Milford, MA).

LC/MS/MS analysis

An aliquot of the tryptic digest (in 2% acetonitrile/0.1% formic acid in water) was analyzed by LC/MS/MS on an LTQ-Orbitrap-XL mass spectrometer (Thermo-Fisher Scientific, Bremen, Germany) interfaced with an Eksigent Nano-LC-Ultra-2D plus CHiPLC Nanoflex system (AB SCIEX, Framingham, MA). A total of 0.5 μg of each sample was loaded onto a ChromXP C_{18}-CL trap column (200-μm inner diameter, 0.5-mm length, 3 μm) at flow rate of 3 $\mu\lambda$/min. Reverse-phase C_{18} chromatographic separation of peptides was carried on a ChromXP C_{18}-CL column (75-μm inner diameter, 15-cm length, 3 μm) at 300 nL/min; column temperature was controlled at 35°C.